

Fig. 1A

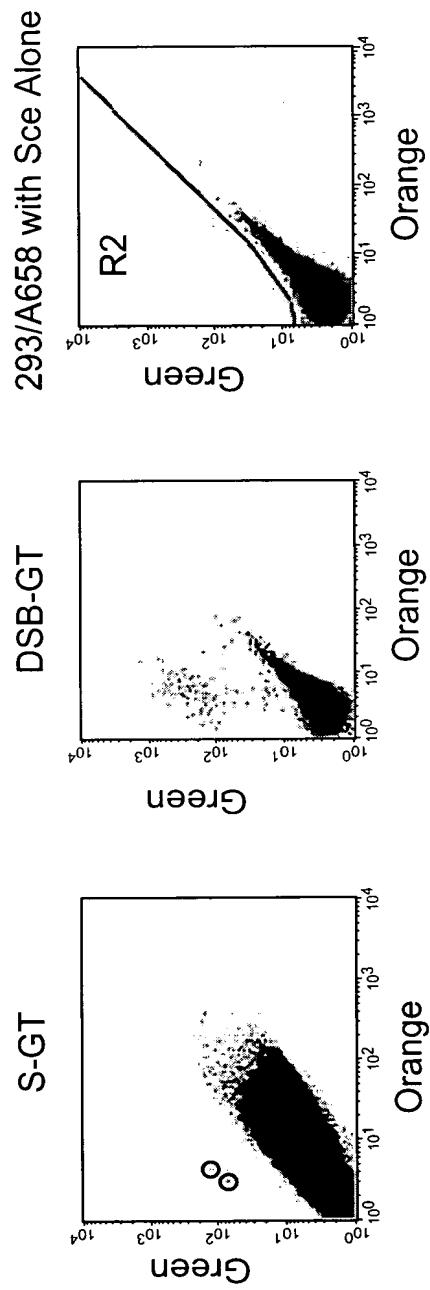


Fig. 1B

Gene Target	1bp insertion		7 bp insertion		35 bp insertion (A658)		35 bp insertion (A658)		66 bp insertion (QQR8)		Fold Stimulation By Sce Induced DSB
	Sce	No									
Events/ 10 ⁶ cells	29.5 +/- 13.0		1.5 +/- 1.1		0.71 +/- 0.27		1600 +/- 500		1900 +/- 250		>2000
Rate	2.9 x 10 ⁻⁵		1.5 x 10 ⁻⁶		7.1 x 10 ⁻⁷		1.6 x 10 ⁻³		1.9 x 10 ⁻³		

Fig. 1C

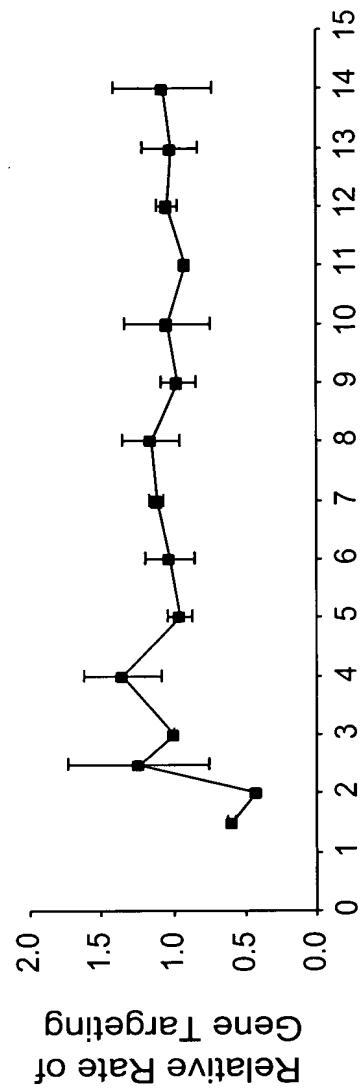


Fig. 1D

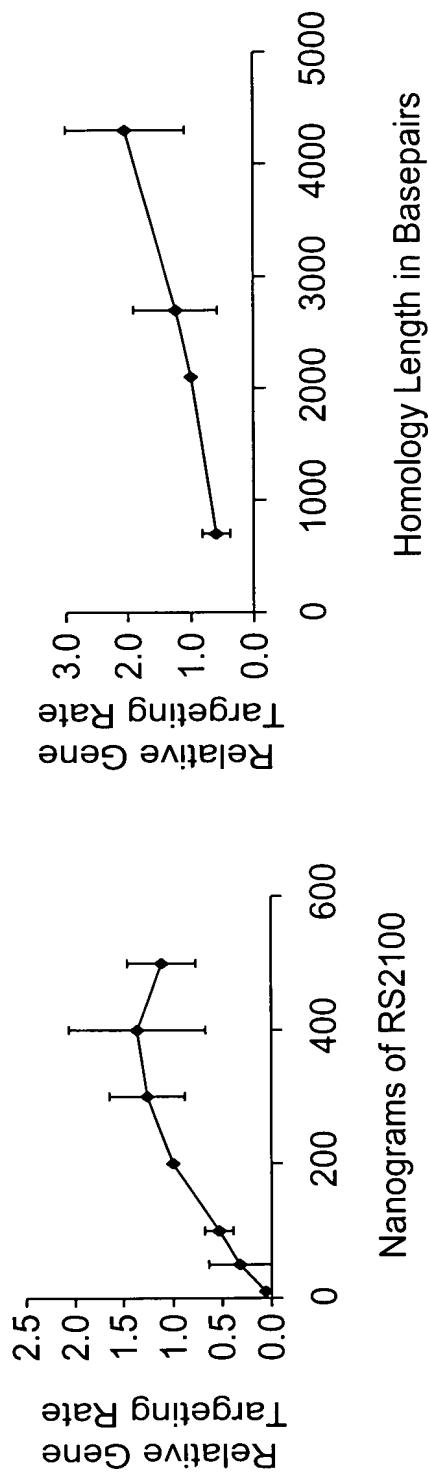


Fig. 2A

Fig. 2B

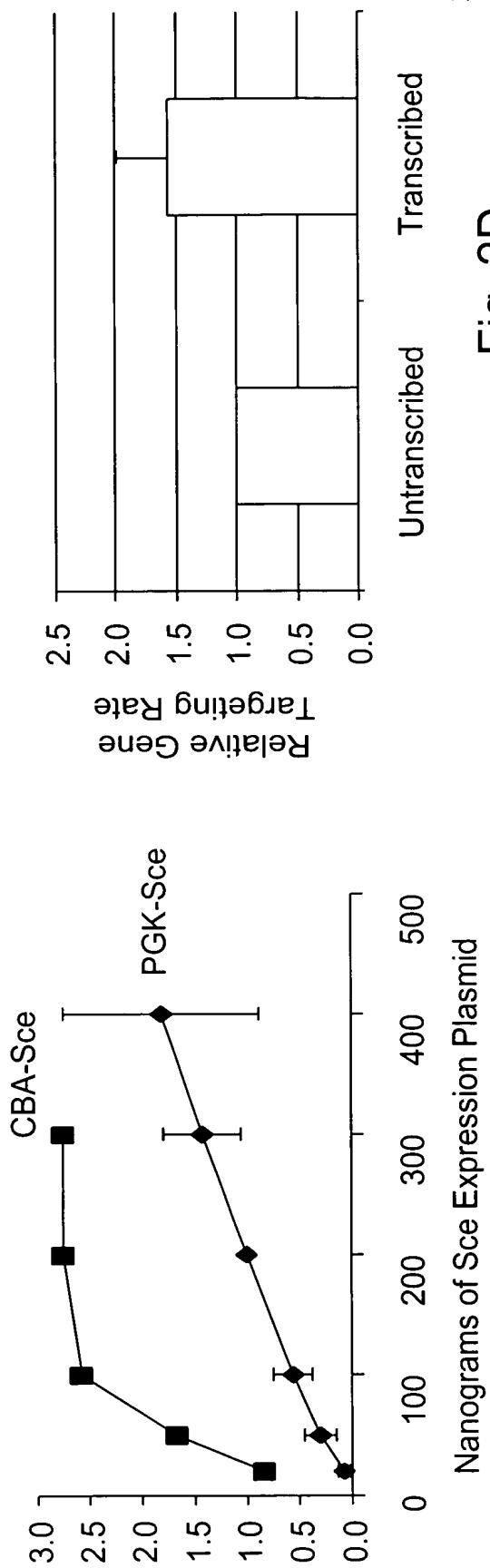


Fig. 2C

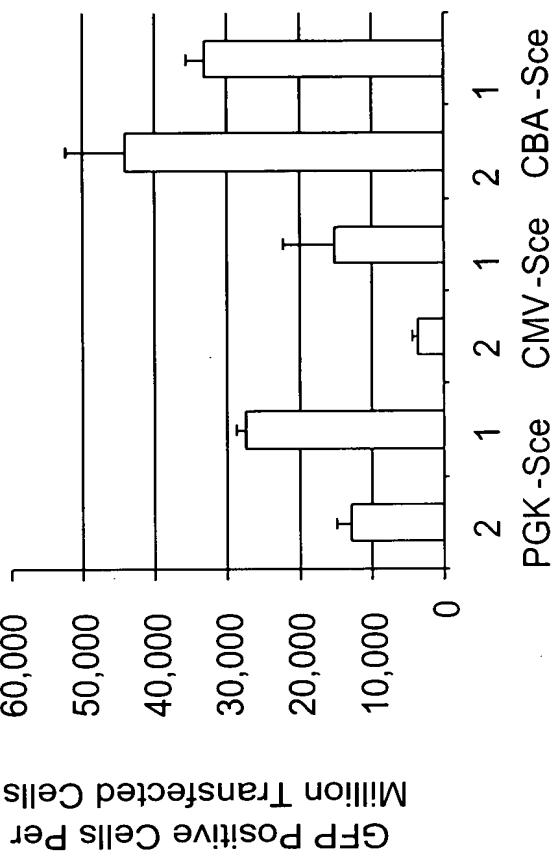


Fig. 2D

Targets:		Chimeric Nucleases:	
QQR8:	5' GFP Stop QQR Site 8 Sce Site 3' GFP	CMV-QQR-L18-Fn:	CMV]ATG[NQQRL18[Nuclease
QQR6:	5' GFP Stop QQR Site 6 Sce Site 3' GFP	CMV-QQR-L0-Fn:	CMV]ATG[NQQRL0[Nuclease
QQRZif6:	5' GFP Stop QQR Site 6 Zif Sce Site 3' GFP	CMV-Zif-L3-Fn:	CMV]ATG[NZif[L3[Nuclease

Fig. 3A

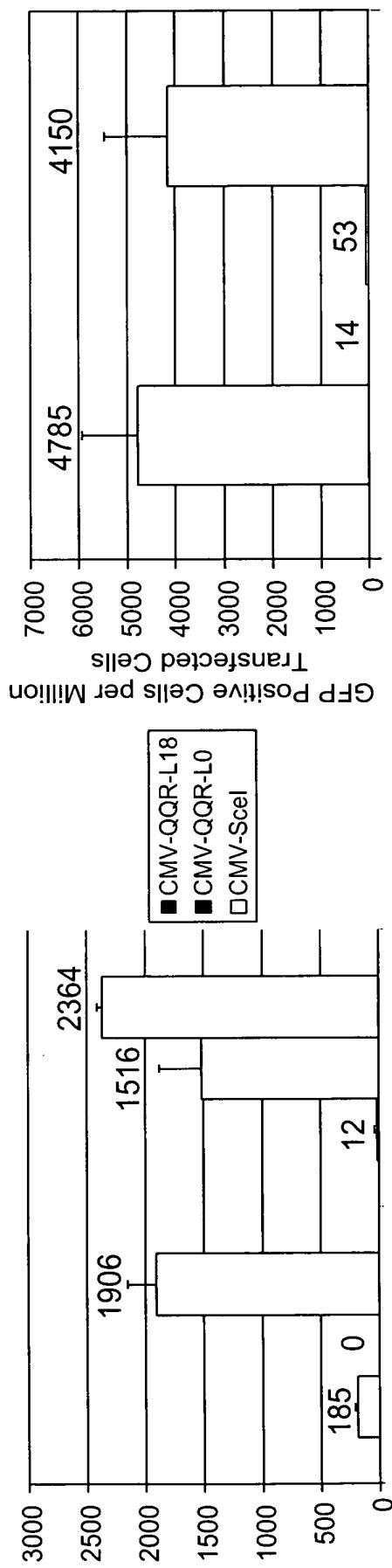


Fig. 3B
QQR6

Fig. 3C
QQR/ Zif

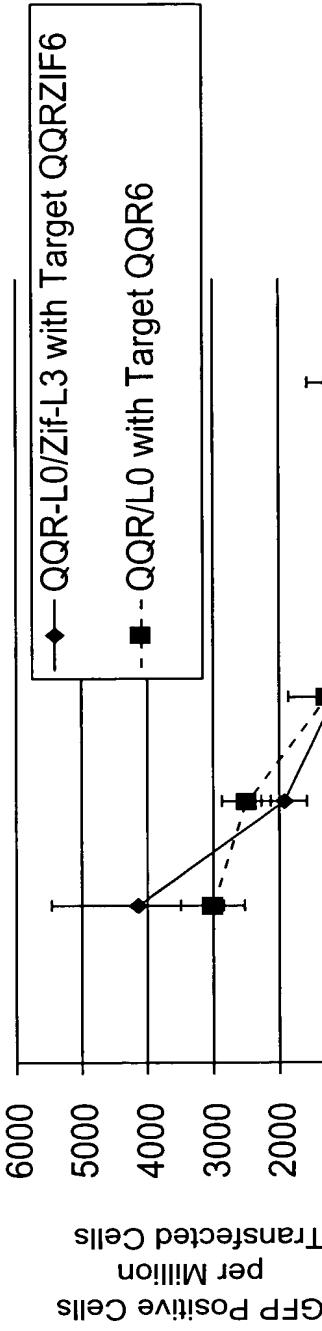


Fig. 3D
Day After Transfection

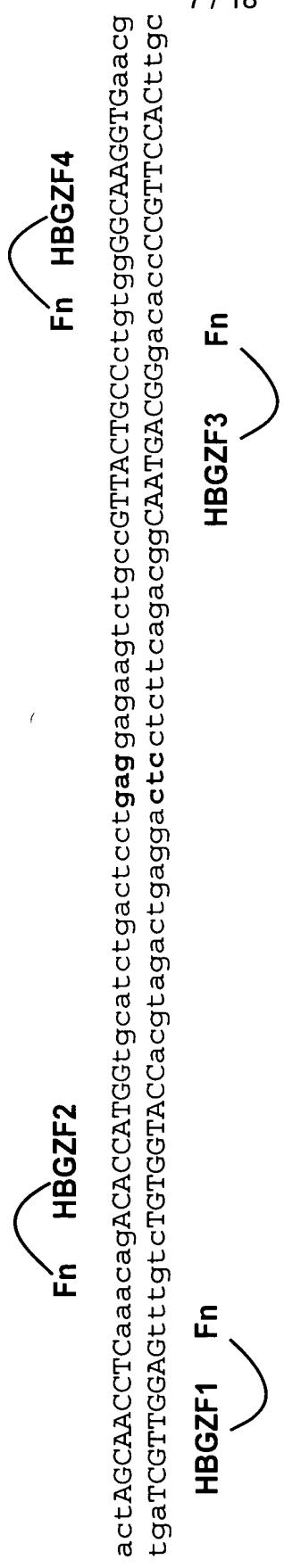


Fig. 4

Design of HBGZF1

Target Binding Site: 5' GAG GTT GCT 3'
F3 F2 F1

		-1	1	2	3	4	5	6
		B3		B2			B1	
Finger 1:	GCT	Q	S	S	D	L	T	R*
		T	S	G	E	L	V	R
Finger 2:	GTT	T	S	G	A	L	T	R*
		T	S	G	S	L	T	R
Finger 3:	GAG	R	S	D	N	L	T	R*
		R	S	D	N	L	V	R

Structure of CMV - HBGZF1

CMV ATG - NLS - FLAG - Finger 1 (QSSDLTR) - Finger 2 (TSGALTTR) - Finger 3 (RSDDNLTR) - Fn

Fig. 5

Gene Targeting with HBGZF1

Target : GFP-Stop-AGCAACCTCtctagaGCGTGGCG-SceSite-GFP
TCGTTGGAGagatctCGCAACCCGC

Fn Zif268

HBGZF1 Fn

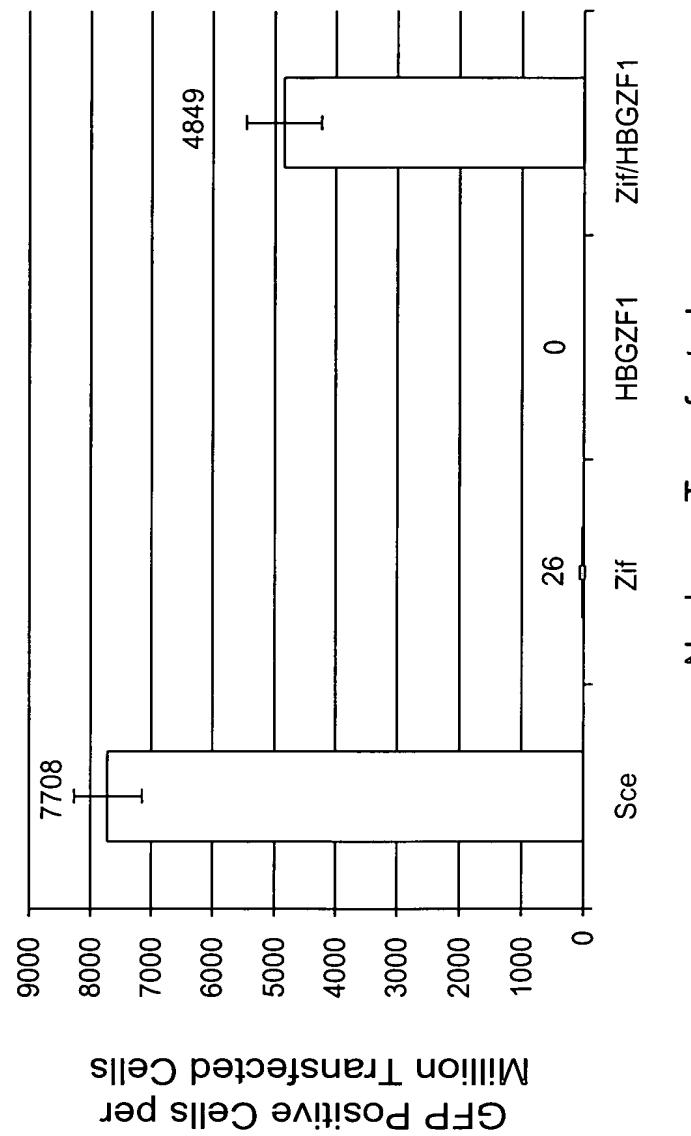


Fig. 6
Nuclease Transfected

Design of HBGZF4

Target Binding Site: 5' GGC AAG GTG 3'

F3 F2 F1

-1 1 2 3 4 5 6
B3 B2 B1

Finger 1: GTG R S D A L T R*
R S N S L T R

Finger 2: AAG R S D T L S N
R K D N L K N
R S S N L T Q*

Finger 3: GGC D R S H L A R*
E S N H L T R
E R S K L A R
D P G H L V R

Structure of CMV-HBGZF1

CMV ATG-NLS-FLAG-Finger 1(RSDALTR)-Finger2(RSSNLTR)-Finger3(DRSHLAR)-Fn

Fig. 7

Gene Targeting with HBGZF4

Target: GFP-Stop-CACCTTGCTcttagAGCGTGGCG-SceSiteGFP
GTGGAACGGagatctCGCAACCGC

HBGZF4 Fn

Fn Zif268

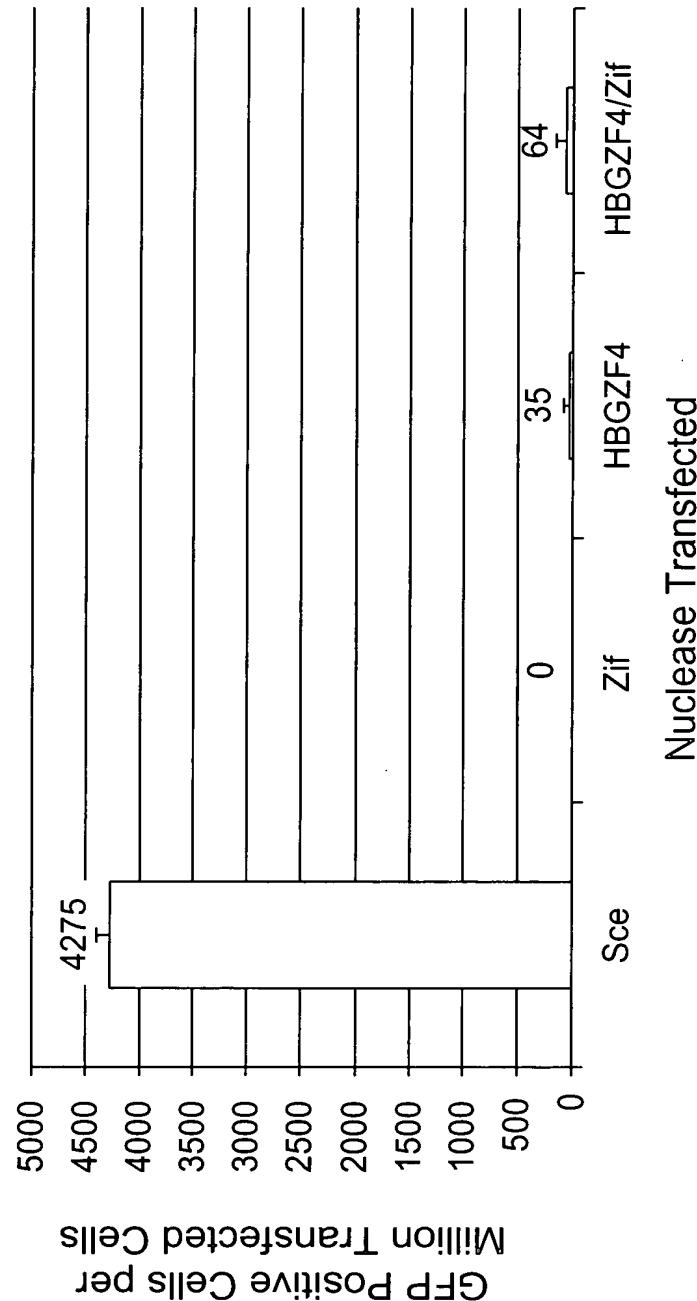


Fig. 8
Nuclease Transfected

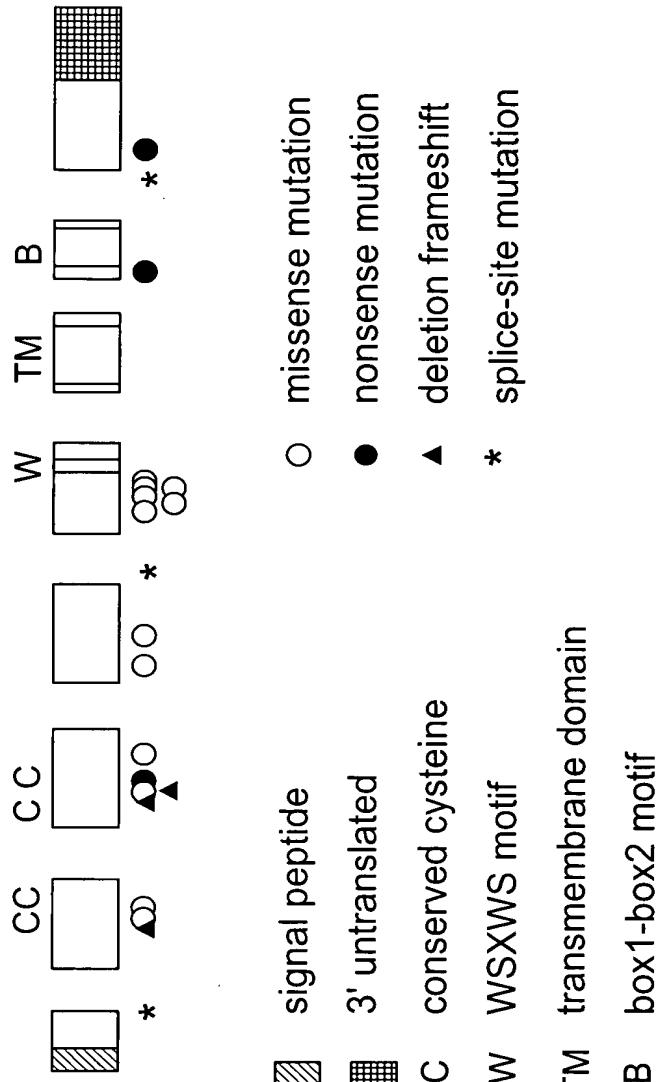


Fig. 9A

Exon 5 5' gaacaatcaggatataagacataaagtttcttccttagtgtggatggcagaaaacgctacacacgtttcggttt
 3' ctttgttagtccacctaataatctgttattcaagaggaaacggatcacacacctacccgtctttgcgtatgtgcaaaaggcaca
WSXWS motif HGCZF2
 cgagccgcgtttaaacccactctgtggaaagtggatggaggccaccaatccactggggtaggttaggtgacccctcggt
 gcctcggcgaattgggtgagacaccttacacgtcgttaacccacttacacttacccctcggt
 atactcaaaaagag 3',
 tatgaaagtttctg 5',

Fig. 9B

Design of HCGCZF2

Target Binding Site: 5' GGG GGA GCA 3'

		F3	F2	F1	
		-1	1	2	3
				4	5
				5	6
B3			B2		B1
Finger 1:	GCA	Q	S	G	S
		Q	S	G	D
		Q	S	G	D
		Q	S	N	D
		Q	S	R	T
					R*
Finger 2:	GGA	Q	S	G	H
		Q	R	A	H
		Q	S	S	H
		Q	S	H	L
					T
					R*
Finger 3:	GGG	R	S	D	H
		R	S	D	H
		R	S	S	H
		R	S	H	L
					T
					R*

Structure of CMV-HCGCZF2

CMV

ATG-NLS-FLAG-Finger 1(QSNDLTR)-Finger2(QSSHLTR)-Finger3(RSSHLTR)-Fn

Fig. 10

Gene Targeting with HCGCZF2

F_n Zif268

Target : GFP-Stop-TGCTCCCCtctagaGGCGTGGCG-SceSite-GFP
ACGAGGGGGagatctCGCAACCGC

HCGCZF2 F_n

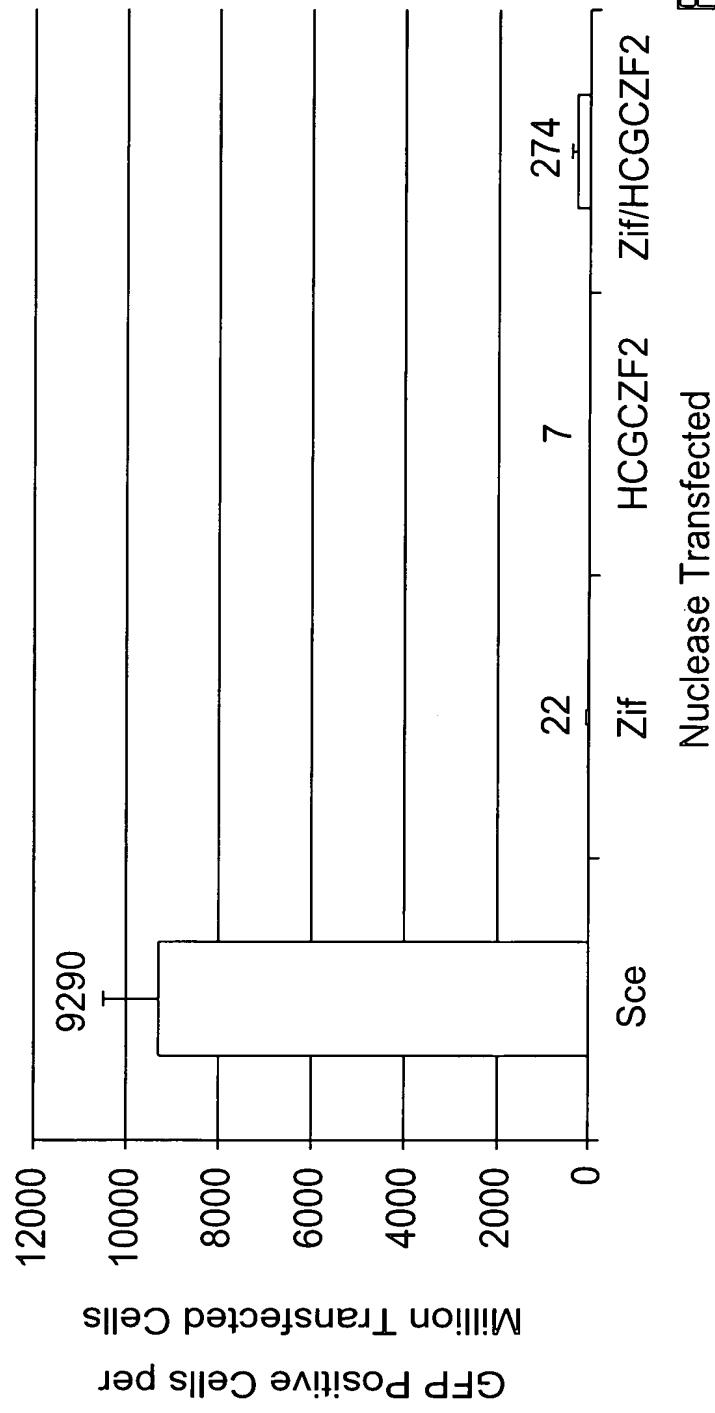


Fig. 11

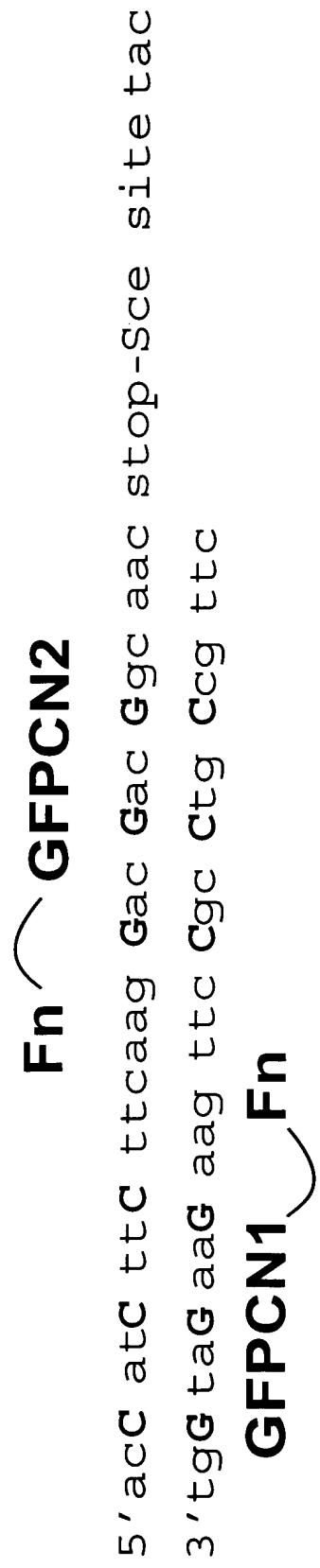


Fig. 12A

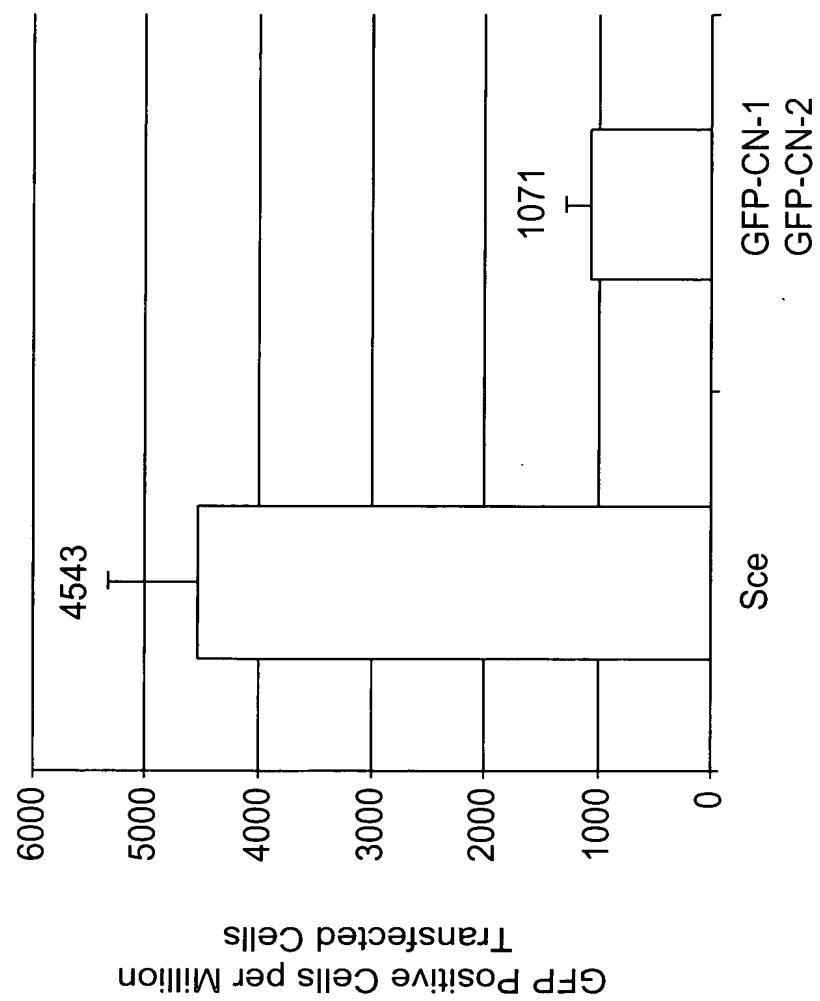


Fig. 12B

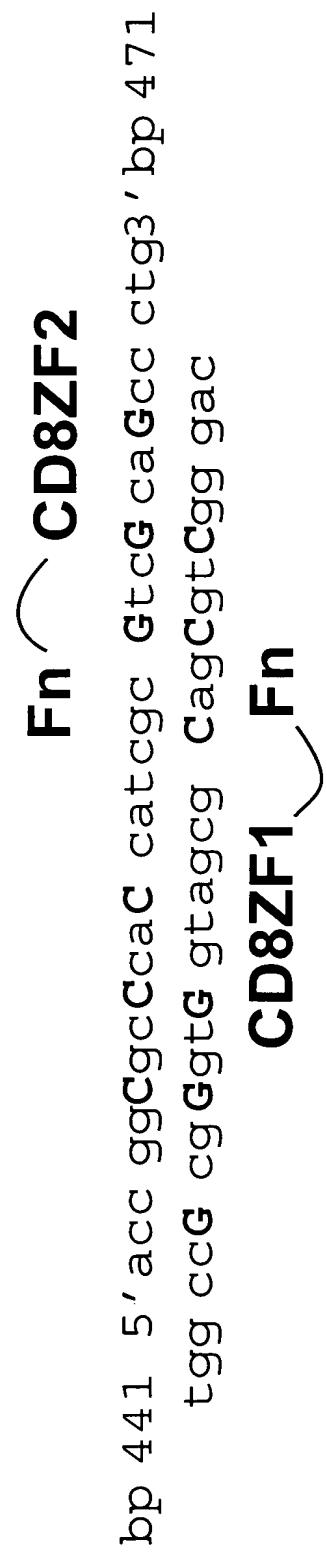


Fig. 13A

Cell Line	CMV/CBA	GFP*	IRES	CD8
293/1104				95% CD8 Positive

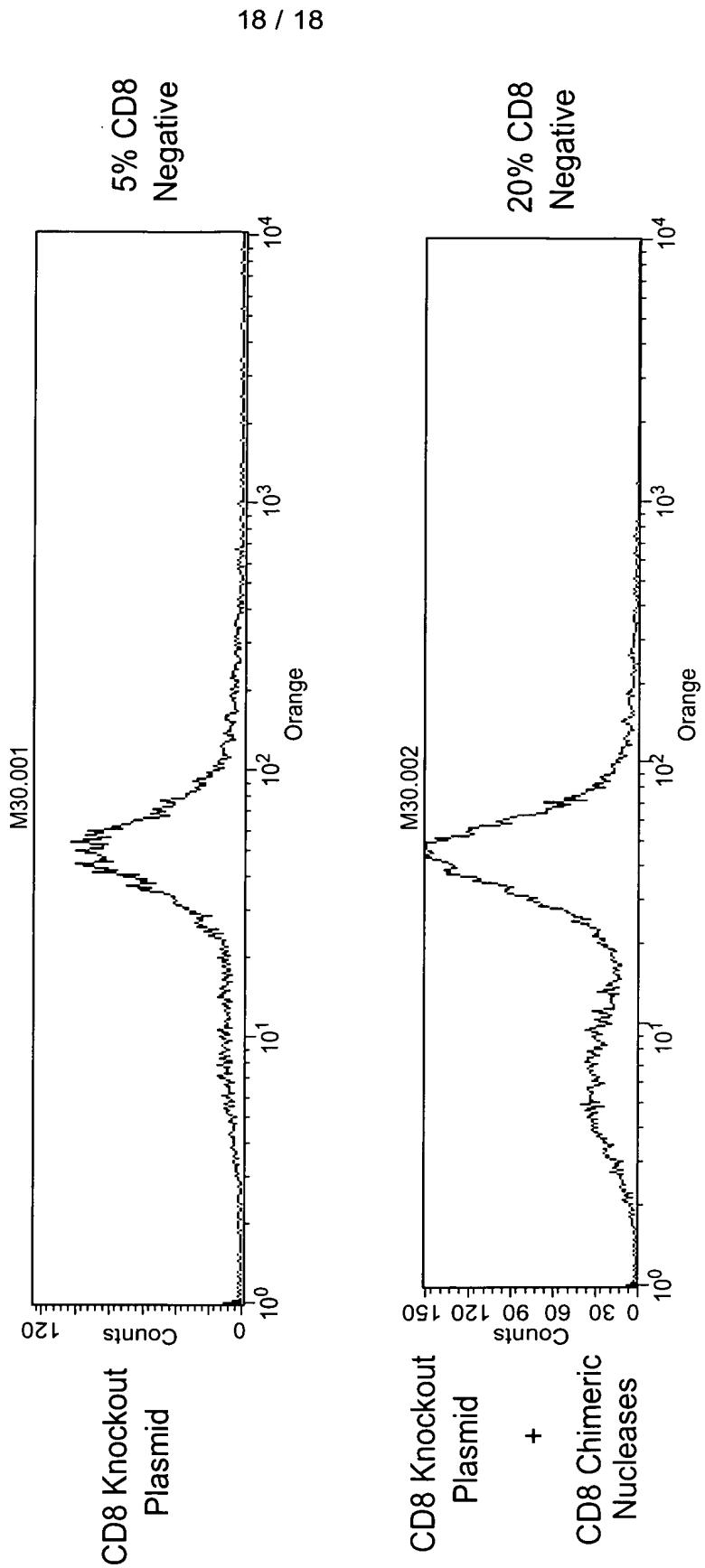


Fig. 13B